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NEW DEVELOPMENTS IN RED BLOOD CELL PRESERVATION  
USING LIQUID AND FREEZING PROCEDURES

by

C. R. VALERI

NAVAL BLOOD RESEARCH LABORATORY  
BOSTON UNIVERSITY SCHOOL OF MEDICINE  
615 ALBANY ST.  
BOSTON, MA 02118

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After storage at 4 C, the red blood cell concentrates can be biochemically treated with a solution containing pyruvate, inosine, phosphate and adenine, to restore or improve the red cell 2,3 DPG and ATP levels. Biochemically modified red blood cells may be cryopreserved for indefinite storage, or they may be transfused immediately after rejuvenation, but whether frozen or not they must be washed before transfusion to remove the potentially toxic substances in the rejuvenation solution. Biochemical modification of outdated universal donor O-positive and O-negative red cells is being used primarily to salvage outdated red blood cells. However, indated red blood cells are also being biochemically modified to increase the 2,3 DPG levels to 2 to 3 times normal and improve oxygen transport function; these red blood cells are useful in the treatment of anemic patients with fixed coronary and cerebral blood flow and for hypothermic patients during cardiopulmonary bypass surgery.

A newly developed polyvinylchloride plastic multiple-bag collection system has simplified blood collection, component separation and storage, biochemical modification with or without cryopreservation, cryopreservation with or without biochemical modification, and pre-wash dilution of the red cells.

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ABSTRACT

Today, most donor blood is separated into its components -- red blood cell concentrates, platelet concentrates, and plasma -- shortly after collection. The storage limitations depend upon which anticoagulant is used during blood collection: whole blood and red blood cell concentrates may be stored at 4 C with hematocrit values of 75 to 80 V% for 21 days in CPD and for 35 days in CPDA-1.

After storage at 4 C, the red blood cell concentrates can be biochemically treated with a solution containing pyruvate, inosine, phosphate and adenine, to restore or improve the red cell 2,3 DPG and ATP levels. Biochemically modified red blood cells may be cryopreserved for indefinite storage, or they may be transfused immediately after rejuvenation, but whether frozen or not they must be washed before transfusion to remove the potentially toxic substances in the rejuvenation solution. Biochemical modification of outdated universal donor O-positive and O-negative red cells is being used primarily to salvage outdated red blood cells. However, indated red blood cells are also being biochemically modified to increase the 2,3 DPG levels to 2 to 3 times normal and improve oxygen transport function; these red blood cells are useful in the treatment of anemic patients with fixed coronary and cerebral blood flow and for hypothermic patients during cardiopulmonary bypass surgery.

A newly developed polyvinyl chloride plastic multiple-bag collection system has simplified blood collection, component separation and storage, biochemical modification with or without cryopreservation, cryopreservation with or without biochemical modification, and pre-wash dilution of the red cells.

Blood is a scarce and expensive product with a relatively short existence. Two major hurdles must be overcome, first to stimulate more people to donate blood, and second to make better use of the collected blood. Many tests have been instituted over the years to enhance the quality of blood transfusion. Some of these include: blood compatibility testing using the ABO and Rh antigenic systems, screening of donor serum for atypical antibodies, crossmatching donor red cells using the anti-globulin test, collection of blood from only volunteer donors, and the introduction of the test for hepatitis B surface antigen.

One problem that has not been resolved though is that of periodic shortages of donors, usually during the periods when blood demands are high. There are periods when blood donations are at an acceptable level, but often this blood sits on the shelf until it becomes outdated because of an imbalance in supply and demand. Within the past 15 years, many blood banks have started freezing red blood cell concentrates during high donation periods.<sup>1</sup> Nevertheless, many units still are lost as a result of outdateding.

The discard of outdated red blood cells has been a frustrating experience for blood bankers trying to keep up with hospital requirements. Now, however, a process of biochemical modification can be employed to salvage the outdated red blood cells. The solution used to biochemically modify the red blood cells contains substances to restore or increase 2,3 DPG and ATP levels which fall during liquid storage. The intelligent use of rejuvenation and cryopreservation processes can virtually eliminate the serious problem of blood wastage, at the same time ensuring a high

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quality product for transfusion therapy.

During liquid storage of red blood cells, 2,3 DPG, the organic phosphate compound, which regulates red cell function, deteriorates rapidly. A process of biochemical modification can be used to increase the 2,3 DPG and ATP levels of stored red blood cells in vitro: the 2,3 DPG level of indated red blood cells can be increased to two to three times normal, and that of outdated red blood cells to normal or 150% of normal. Rejuvenated red blood cells may be transfused immediately or they may be cryopreserved for indefinite storage. Whether frozen or not, rejuvenated red blood cells must be washed before transfusion to remove the potentially toxic substances in the rejuvenation solution. Caution must be taken to avoid contamination during addition of the rejuvenation solution and during red cell washing.

#### Use of Red Blood Cells and Resuscitative Fluids in the Treatment of Hemorrhagic Shock

Crystalloid and colloid solutions often are used in the resuscitation of blood volume and interstitial tissue fluid volume in hemorrhagic shock: colloid solutions are far more expensive than crystalloid solutions.<sup>2-5</sup> The colloid solutions include: fresh frozen plasma, 5% albumin solution and albumin concentrates, plasma protein fraction (PPF), hydroxyethyl starch (HES), dextran (high molecular weight (HMW) and low molecular weight (LMW)), and the gelatin-like

solutions, physiogel, gelifundol, plasmagel, and haemacel.<sup>6</sup> Albumin, with which hepatitis has not been a problem, is a highly potent oncotic protein and an excellent substance for increasing plasma volume, although its retention in patients is shortlived. The side effects associated with the use of albumin in hemorrhagic shock include: pulmonary, renal, and cardiac insufficiency, and impairment of the blood clotting mechanism.<sup>7-10</sup>

Crystalloid solutions, given in volumes two to three times those of colloid solutions, have been used successfully in the treatment of early hemorrhagic shock, and the concern that these large volumes might precipitate acute pulmonary insufficiency appear to be unfounded.<sup>2-5</sup> Isotonic and hypertonic (7.5 percent) sodium chloride solutions with and without glucose, and Ringer's solution with and without lactate also have been used.<sup>2-5,11</sup>

Red blood cells also have been used, in combination with crystalloid solutions, to treat hemorrhagic shock.<sup>12,13</sup> It is the red cell component of the blood and not the plasma that is needed during moderate or severe hemorrhagic shock. Red blood cells do not themselves have an oncotic effect in vitro but they do produce an in vivo increase in plasma volume, apparently by the mobilization of interstitial albumin, and produce an immediate increase in red blood cell volume followed by a prompt and satisfactory increase in plasma volume.<sup>13</sup> The red blood cells should have satisfactory oxygen transport function to ensure an ample supply of oxygen to tissue.<sup>1</sup> This means that the red blood cells should have normal or increased 2,3 DPG levels at the time of transfusion.

### Component Therapy

Separation of the various components within 4 to 8 hours of blood collection was fairly rare just 20 years ago, but today it is recognized as an intelligent alternative to storage of whole blood.<sup>1</sup> Newly developed polyvinyl chloride (PVC) plastic multiple-bag collection systems make it easy and safe to prepare blood components with virtually no risk of air embolism or contamination sometimes a problem with glass containers. The red cell concentrate is prepared and stored in the primary bag of the collection system, and depending on the number of integrally attached transfer packs, a platelet concentrate, fresh frozen plasma, cryoprecipitate or cryoprecipitate-poor fresh frozen plasma, may be prepared (Figure 1).

FIG. 1

Because this new multiple-bag collection system is made of polyvinyl chloride (PVC), there has been some concern that the potentially toxic plasticizers in PVC, di-2-ethylhexyl phthalate (DEHP) and monoethylhexyl phthalate (MEHP), may leech into the blood products during storage at 4 C or 22 C.<sup>14-23</sup> DEHP in very large concentrations has been reported to be carcinogenic in rodents.<sup>24</sup> Studies have shown though that the accumulation of DEHP in human whole blood usually is about only 1 mg% per day of storage and even less in a red blood cell concentrate, and that red cell washing reduces the DEHP concentration to less than 1 mg%.<sup>1,17,18,20</sup> DEHP measured in platelet concentrates after storage for 3 days at  $22 \pm 2$  C was about 45 mg%.<sup>1,17,18</sup> Plastic containers that do not contain DEHP are being investigated as a possible replacement for the DEHP-containing polyvinyl chloride plastic containers.

The PVC multiple-bag collection system can be obtained with one, two or three transfer packs integrally attached to the primary bag via plastic tubing.

Anticoagulants for Liquid Preservation of Red Blood Cells

Whole blood may be stored at 4 C in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) for 21 days, or in a CPD anticoagulant supplemented with adenine and additional glucose (CPDA-1) for 35 days (Figure 2). Storage of the whole unit of blood in an anticoagulant, however, does not ensure preservation of the whole unit of blood. These anticoagulants do what they were intended to, i.e., they preserve the viability of the red blood cells. It was not intended that they should preserve red cell function, platelet viability or function, granulocyte viability or function, or plasma labile clotting proteins, and they do not. The plasma oncotic proteins (albumin, gamma globulin, and fibrinogen) and the plasma opsonic proteins (the IgG immunoglobulins and complement) are preserved for about 3 weeks of blood storage in ACD or CPD at 4 C.<sup>1</sup> A red cell concentrate prepared within 4 to 8 hours of blood collection has storage limitations similar to those of whole blood. The platelet concentrate can be stored at 4 C for 48 hours or at 22 C for 3 days, and the fresh frozen plasma and cryoprecipitate can be frozen and stored at -20 C.<sup>1</sup>

FIG. 2

The ACD anticoagulant is not as popular as it once was; CPD usually is used because it provides better maintenance of the red cell 2,3 DPG level. More recently, the CPD anticoagulant has been supplemented with adenine and additional glucose to extend the shelf life of the red

cells.<sup>25-28</sup> Red cells now can be stored at 4 C for as long as 35 days in the newly licensed CPDA-1 anticoagulant, and in studies of two Investigational New Drugs, CPDA-2 and CPDA-3, red cells have been stored for at least 42 days with satisfactory results. The larger concentrations of adenine and glucose in the supplemented CPD anticoagulants maintain the red cell ATP level for longer periods; however after an initial increase in 2,3 DPG during the first 48 hours subsequent deterioration proceeds at an accelerated rate (Figures 3 and 4). Furthermore, although CPDA-1 anticoagulant is supposed to prolong the shelf life of the red cells at 4 C for 35 days, we observed unacceptable 24-hour posttransfusion survivals in CPDA-1 red cells stored with hematocrit values of  $80 \pm 8$  V% for 35 days (Figure 5). Other investigators have reported that survival was maintained in CPDA-1-stored red blood cells for only 28 days.<sup>27</sup>

FIG. 3  
FIG. 4

FIG. 5

We found that when red blood cells were stored in CPD at 4 C, the hematocrit value of the red blood cell concentrate influenced both function and viability, although in different directions. The higher the hematocrit value of the stored red cell concentrate, the better 2,3 DPG is maintained but the poorer the posttransfusion survival value (Figure 6). It should be noted though that whatever the increase in 2,3 DPG during the first 2 weeks of storage, the level begins to fall rapidly after this time, and after 3 weeks of storage at 4 C the 2,3 DPG level is only about 10% of normal. It has been reported that mixing the red blood cells periodically during 4 C storage improves maintenance of 2,3 DPG, but we did not observe this

FIG. 6

in our laboratory. Thus, although supplemented CPD anticoagulants, CPDA-2 and CPDA-3, maintain the viability of red cell concentrates with a hematocrit value of  $80 \pm 5$  V% at 4 C for at least 42 days, the oxygen transport function of these red cells is impaired after only about 10 days.

#### "Cold" and "Warm" Rejuvenation

"Cold" rejuvenation during liquid storage and "warm" rejuvenation after liquid storage have been used by numerous researchers to maintain or restore the quality of liquid red blood cells. Cold rejuvenation during storage at 4 C ensures maintenance of ATP and 2,3 DPG for longer periods, and the treated red cells have acceptable posttransfusion survival and normal or improved oxygen transport function at the time of transfusion. Inexpensive docking systems are needed to add the rejuvenation solution to avoid contamination which might occur if the closed system is entered during addition of the solution.<sup>29</sup> Hogman and associates have reported on an aseptic method of adding the rejuvenation solution directly to the red cell concentrate in the primary bag through an integrally attached transfer pack of a multiple-bag collection system.<sup>25</sup>

Various combinations of substances have been used for cold rejuvenation, including adenine, inosine, pyruvate, guanosine, glucose, phosphate, dihydroxyacetone, ascorbate, methylene blue, phosphoenolpyruvate, and phosphate ion exchange resin.<sup>30-34</sup> Solutions of bicarbonate, adenine, glucose and mannitol (BAGM), saline, adenine and

glucose (SAG), and adenine, glucose, sodium chloride and mannitol (ADSOL), have been used to maintain or increase the red cell organic phosphate compounds, ATP which influences posttransfusion survival, and 2,3 DPG which influences red cell oxygen transport function. Studies have shown that mannitol inhibits red cell hemolysis, that ATP usually deteriorates more slowly at a slightly acidic pH, and that there usually is less deterioration of 2,3 DPG at a slightly alkalotic pH.

Red blood cells have been treated by incubation with a rejuvenation solution in a 37 C water bath for 1 to 3 hours.<sup>35-37</sup> The longer the period of incubation at 37 C, the greater the potential for contamination. A 1-hour period of incubation at 37 C after adding the solution should ensure the sterility of the rejuvenated red cells, although another potential for contamination may arise during red cell washing, which is essential to remove the rejuvenation solution before transfusion.

Cold or warm rejuvenation, either to maintain or restore ATP and 2,3 DPG levels, is only a stop-gap measure. If rejuvenated red blood cells are stored in the liquid state, deterioration of ATP and 2,3 DPG will again progress. Rejuvenated red cells can only be stored for extended periods if they are frozen immediately after rejuvenation. Indated-rejuvenated and outdated-rejuvenated red cells have been frozen with 20% or 40% W/V glycerol, and after thawing and washing have been used extensively in clinical studies conducted by the Naval Blood Research Laboratory. When the newly developed polyvinyl chloride plastic multiple-bag collection systems are used, the red blood cells must be frozen by the

high glycerol method. Polyvinyl chloride plastic containers do not withstand the low temperature of liquid nitrogen used with the low glycerol freezing method.

Physiologic Importance of Oxygen Transport Function of Preserved Red Cells

It has been more than 25 years since Valtis and Kennedy<sup>38</sup> first described the impairment of oxygen transport function in red blood cells after storage at 4 C, and almost 15 years since Benesch and Benesch<sup>39</sup> and Chanutin and Curnish<sup>40</sup> reported that this respiratory defect was due to deterioration of red cell 2,3 DPG occurring during liquid storage of red blood cells at 4 C. The correction of this impairment in vivo, usually within 24 hours after transfusion, later was shown to correlate with restoration in vivo of red cell 2,3 DPG.<sup>41-43</sup> Several factors in the recipient's health were found to influence the rate of in vivo synthesis of 2,3 DPG: acid-base status, degree of anemia, cardio-pulmonary function, plasma inorganic phosphorus level, and other factors.

From 1968 to 1972 the Naval Blood Research Laboratory was involved in studies of more than 300 patients who had sustained war injuries in South Vietnam and were being treated at the Chelsea Naval Hospital, later named Boston Naval Hospital, Chelsea, Massachusetts. These patients were brought to our attention when the use of general anesthesia for routine debridement of wounds precipitated a life-threatening state of hypotension. The observation was made that these patients with reduced red blood cell volumes compensated for this reduction by increasing their red blood cell 2,3 DPG levels to ensure optimum oxygen transport function without an increase in cardiac output. A patient



usually compensates for a 40% reduction in the red blood cell volume by increasing the level of 2,3 DPG from 0.8 M DPG per M Hb or 13  $\mu\text{M/g}$  Hb to 1.6 M DPG per M Hb or 26  $\mu\text{M/g}$  Hb, which is 2 times the normal level.<sup>44</sup>

The observation of this compensatory increase in red cell 2,3 DPG to improve oxygen transport function led us to experiment with solutions to produce in vitro elevations in the 2,3 DPG levels of liquid-stored red blood cells. The rejuvenation solution being used at that time was the PIGPA solution, containing pyruvate, inosine, glucose, phosphate, and adenine.<sup>1</sup> Both the high and low glycerol freezing methods were used, with freezing containers composed of inert bioriented polyolefin or PVC plastic. After storage in the frozen state for as long as 2 years, these biochemically treated red blood cells had normal or improved oxygen transport function and greater than 70% posttransfusion survival values.<sup>1</sup>

#### Rejuvenation of Indated and Outdated Red Blood Cells Prior to Cryo-Preservation

Red blood cell ATP is synthesized from adenine and phosphate and red blood cell 2,3 DPG is synthesized from the inosine, pyruvate and phosphate in the rejuvenation solution. The pH of the rejuvenation solution influences the rate at which the phosphate enters the red cell. The rejuvenation process restores only the red cell 2,3 DPG and ATP levels so it is recommended that the red cell component be separated within 4 to 8 hours of blood collection. Whether or not rejuvenated red cells are frozen, they must be washed before transfusion to remove the

potentially toxic substances.<sup>45-48</sup> The basic principles of cryopreservation have been reported elsewhere.<sup>1</sup>

To summarize briefly, human red cells are currently being frozen either with a high concentration of glycerol (40% W/V) and storage at -80 C in mechanical refrigerators or with a low concentration of glycerol (20% W/V) and storage at -150 C in the gas phase of liquid nitrogen. With recent improvements in the high glycerol concentration method, it is now possible to wash the thawed red cells with only 1.5 liters of wash solution instead of the previously used 3.2 liters; this is possible because the supernatant glycerol is removed from the red cells prior to freezing. Cryopreservation with 40% W/V glycerol has been simplified even further by the development of a multiple-bag collection system in which the primary bag is used for blood collection, component separation, red cell glycerolization with or without prior rejuvenation, removal of the supernatant solution prior to freezing, freezing, thawing and post-wash dilution.

The Northeast Red Cross Blood Center has obtained a license from the Bureau of Biologics to use the FRES solution, manufactured by Fenwal Laboratories, to rejuvenate liquid-stored red blood cells as part of their frozen blood banking system. FRES is a liquid solution of inosine and phosphate in a tromethamine buffer, with a separate bottle of pyruvate that is lyophilized and added to the liquid solution at the time of red cell rejuvenation. Another solution, PIPA Solution C, developed at the Naval Blood Research Laboratory, is now being manufactured by PIPA Laboratories, Roslindale, Massachusetts. Unlike the FRES solution, PIPA Solution C contains adenine which produces a greater increase in red cell ATP. The ATP level remains increased even after freezing, thawing, and

washing, so that the red cells have higher posttransfusion survival values after post-wash storage at 4 C. The U. S. Navy expects PIPA Solution C to be licensed in the near future: an application will be submitted by the Blood Bank at the National Naval Medical Center in Bethesda, Maryland for the rejuvenation and cryopreservation of red cells in the 800 ml primary bag of the newly developed PVC multiple-bag collection system.

In our early rejuvenation studies, we used a PVC multiple-bag collection system with a 600 ml primary bag. The primary bag was used for red cell storage at 4 C, rejuvenation, and partial glycerolization, but not for freezing. The partially glycerolized red cells were transferred to a special container for completion of the glycerolization process, freezing and storage at -80 C.<sup>1</sup> The supernatant of the glycerol solution was frozen with the unit and removed during post-thaw washing of the red cells. With the 600 ml primary bag we had a problem with damage to the red cells during glycerolization, but this problem has been reduced with the use of a collection system with an 800 ml primary bag (Figure 7). The 800 ml primary bag containing the anticoagulant is used for blood collection, red cell concentrate preparation and storage at 4 C; rejuvenation, glycerolization to 40% W/V, freezing, and post-thaw dilution.<sup>49,50</sup> The transfer packs that are integrally attached to the primary bag are used for preparation of a platelet concentrate and one other blood component. The remaining empty transfer pack has been used for collection of the supernatant of the rejuvenation and glycerol solutions from the red blood cell concentrate before freezing. Removal of the supernatant before freezing makes the frozen unit smaller so that a greater number of units can be stored in each freezer. The hematocrit of the unit at the time of freezing

should be approximately 65 V%.

The frozen unit in the primary bag is overwrapped in a sealed plastic bag, placed in a cardboard freezing box, and frozen by storage in a -80 C mechanical freezer. Frozen outdated-rejuvenated red cells may be stored at -80 C for at least 4 years.

Any amount of handling of blood after collection carries with it a risk of contamination.<sup>1</sup> This has always been a concern with cryopreservation, and the risk may increase when the rejuvenation process is included. The new freezing protocol described was developed with the contamination risk in mind. The red blood cell concentrate remains in the sterile environment of the primary bag throughout the rejuvenation and cryopreservation processes. It is only after pre-wash dilution of the red cells that the primary bag is opened and the diluted red cells are removed for washing.

The Haemonetics Blood Processor 115 and the IBM Blood Processor 2991-1 and 2991-2 washing systems have been adapted to our new protocol with good results (Figure 8). Details on the principles of red cell washing and on the current methods of red cell washing have been reported elsewhere.<sup>1</sup> The Haemonetics Blood Processor 115 and the IBM Blood Processor 2991-1 and 2991-2 are the systems used at the Naval Blood Research Laboratory to wash non-rejuvenated and rejuvenated liquid-stored and non-rejuvenated and rejuvenated previously frozen red cells. The Haemonetics Blood Processor 115 is a continuous-flow centrifugation cell washing system using disposable rigid polycarbonate bowls. The IBM Blood Processor 2991-1 or 2991-2 is an automated serial centrifugation

washing system which utilizes plastic washing bags.

The red cells are washed with a 50 ml volume of a solution of 12% sodium chloride and 1.5 liters of a solution containing 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus, pH 6.8. The 40 mg% inorganic phosphorus in the solution increases the osmolality to 340 mOsm/kg H<sub>2</sub>O, and helps maintain the red cell 2,3 DPG and ATP levels.

In the Haemonetics Blood Processor 115, two units of red cells can be washed in the same disposable bowl using a single harness with a stylette for each unit, but only if both units are transfused to the same patient. A special 4-bag system is used for this washing process: one bag for collection of each of the washed units, and one bag for collection of the supernatant from each of the units after concentration by centrifugation at the time of transfusion.

Red cell washing removes the glycerol cryoprotectant, the white blood cells, platelets and plasma protein, citrate, and any DEHP that may have leached into the red cells during liquid storage in the plastic container.

Although we have described above the method of washing previously frozen non-rejuvenated and rejuvenated red blood cells, it is important that we stress again that rejuvenated red blood cells must be washed before transfusion whether or not they are frozen to remove the potentially toxic substances in the rejuvenation solution.

It is a blood banking rule that previously frozen red cells not be stored at 4 C for longer than 24 hours after sterile opening of the unit.

However, our laboratory has collected data to indicate that this conservative approach is not necessary. We have studied non-rejuvenated and rejuvenated previously frozen red cells after storage in the final wash solution at 4 C for 3 days after washing and have found them to have normal or slightly improved oxygen transport function and acceptable posttransfusion survival values. Bacteriologic studies made on these red blood cells were negative for contamination.

Once a blood bank decides to become involved in red cell rejuvenation, the newly developed PVC plastic multiple-bag blood collection system should be incorporated into their program. The number of transfer packs integrally attached to the 800 ml primary bag would depend on the number of components the blood bank intends to prepare (Figure 9). The simplest system, of course, would be a single transfer pack attached to an 800 ml primary bag by plastic tubing with an adaptor port (Figure 10). These plastic bag systems are now available from Fenwal Laboratories, Deerfield, Illinois.

FIG. 9

FIG. 10

Cryopreserved red cells may or may not be clinically superior to liquid-stored whole blood; there has been some disagreement over this point. However, cryopreservation as a means of eliminating blood shortages certainly would offer several benefits to the liquid blood banking system. The sensible approach would be to stockpile non-rejuvenated and indated- and outdated-rejuvenated cryopreserved universal donor O-positive and O-negative red cells, rare red cells,

and selected red cells lacking antigens that commonly sensitize recipients (Table 1). Red cells that would normally be discarded when they have reached their outdating period could be rejuvenated and frozen, thereby eliminating much of the blood wastage that occurs today. Outdated red blood cells may be transfused immediately after rejuvenation, but they must be washed first.

TABLE 1

Red cells biochemically treated after 6 to 8 days of storage at 4 C have 2,3 DPG levels increased to 2 to 3 times normal and improved oxygen transport function upon transfusion. Improved oxygen transport function is especially important in the treatment of patients with fixed cerebral and coronary blood flow, hypothermic patients in hemorrhagic shock, and hypothermic patients undergoing cardiopulmonary bypass surgery. When outdated red cells are biochemically treated, they have 150% of normal 2,3 DPG and improved oxygen transport function, and acceptable posttransfusion survival.

The rejuvenation and cryopreservation processes offer blood banks a considerable degree of flexibility. An integrated system incorporating both liquid preservation and cryopreservation of non-rejuvenated and rejuvenated red cells would ensure the availability of high quality universal O-positive and O-negative donor red cells for use during emergency situations and for low donation periods. Approximately 20% of all red cell requirements could be met from a stockpile of frozen red cells, with a good portion of the stockpile consisting of outdated-rejuvenated frozen O-positive and O-negative red cells.

Studies conducted at Navy military facilities have shown that a Frozen Blood Bank Module can be set up to supplement the liquid blood banking system in a combat zone (Figure 11). Such a facility could provide about 20% of the red cell requirements (frozen non-rejuvenated universal donor O-positive and O-negative red cells with storage at -80 C for at least 10 years, and frozen outdated-rejuvenated universal donor O-positive and O-negative red cells with storage at -80 C for at least 4 years), 100% of the platelet needs (with cryopreserved platelets frozen with 6% DMSO and stored at -80 C for at least 1 year), and 100% of the labile clotting proteins (in the form of fresh frozen plasma and cryoprecipitate with storage at -20 C for 1 year).

FIG. 11

As part of this military feasibility study, the Naval Blood Research Laboratory prepared a cost analysis of the establishment and operation of a frozen blood bank: the costs cited do not include the salaries of personnel or the amortization of equipment. The total initial cost for hardware to store 1,000 units of frozen red cells and 100 units of frozen pooled platelets is approximately \$100,000.00. The following hardware is needed: 4 air cooled mechanical freezers with a capacity sufficient to freeze and store 1,000 units of red cells and 100 pools of platelets (each pool containing 6 to 8 units of platelets); 8 Haemonetics Blood Processor 115 cell washers; 2 modified Eberbach shakers; 2 refrigerated centrifuges; and 2 water baths for red cell rejuvenation, glycerolization, and deglycerolization. This hardware is sufficient to rejuvenate, glycerolize and freeze 16 units every 4 hours and to wash



16 units every hour.

The cost of software needed to prepare frozen non-rejuvenated O-positive and O-negative red cells is \$32 to \$38 per unit. The cost of the software to prepare frozen O-positive and O-negative rejuvenated red blood cells is \$38 to \$44 per unit. The cost of the software to prepare frozen pools of 6 to 8 units of platelets obtained from units of whole blood is about \$15 per pool.

In the last analysis, cost continues to be uppermost in the minds of blood bankers when considering any innovation in transfusion practices. It has taken almost 20 years for red blood cell cryopreservation to be accepted by major blood banks as a supplement to their liquid system, not only to build a stockpile of O-positive and O-negative red blood cells, autologous red blood cells, and rare type red blood cells, but also as a means of preserving red blood cells during periods of high donation. Many investigators who initially thought the cost of red blood cell cryopreservation excessive now concede that it actually costs no more to rejuvenate a unit of outdated red blood cells than it does to draw a new unit of blood to replace the discarded outdated unit. The fact that non-rejuvenated and rejuvenated cryopreserved red blood cells properly prepared are of superior quality strengthens the case for adding these processes to current blood banking practices.

FIGURE 1

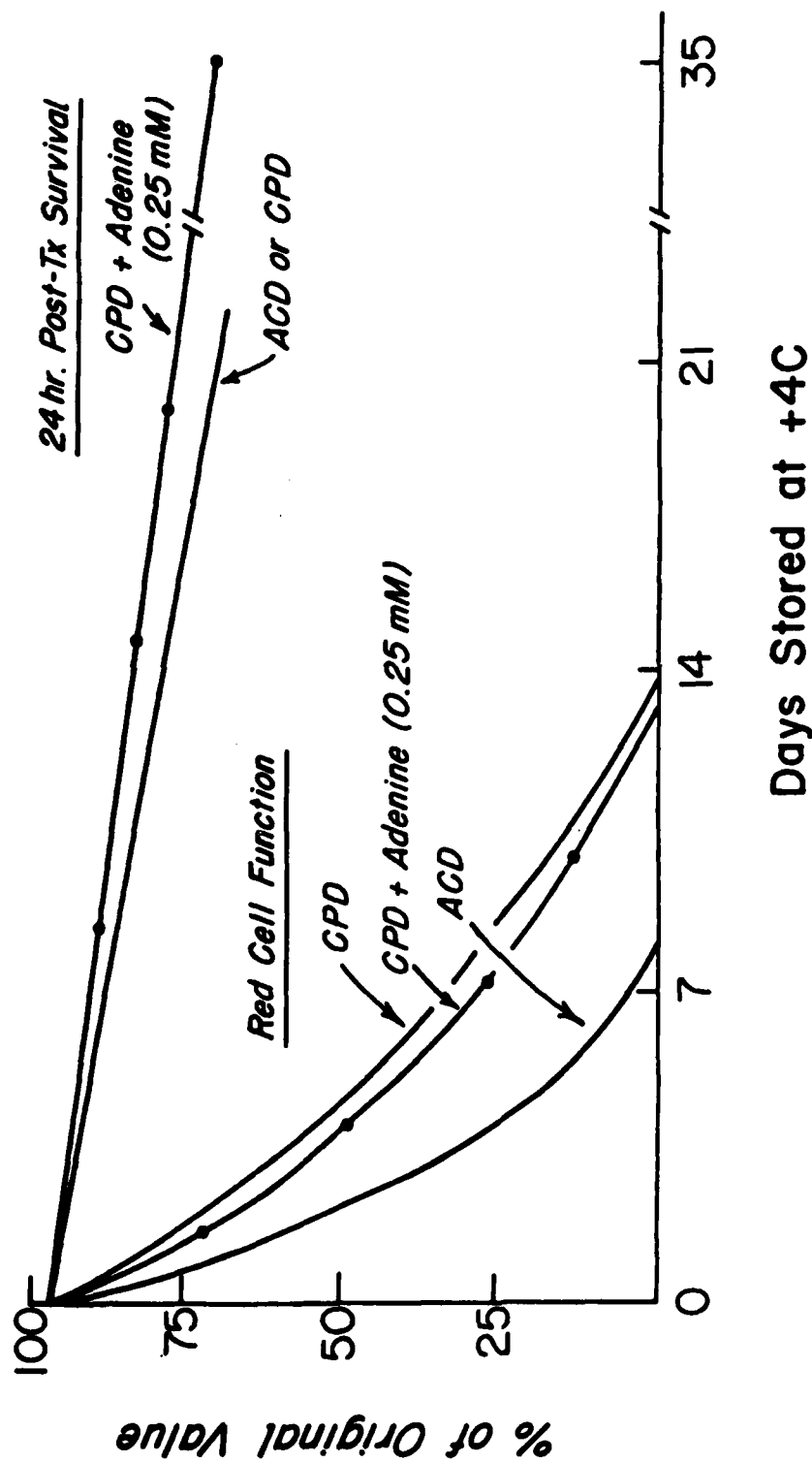
An approach to collecting 450 ml of blood and separating it into cellular components and plasma protein derivatives. The red blood cell and platelet concentrates and plasma protein derivatives are isolated from blood within 4 to 8 hours of collection and storage at room temperature ( $22 \pm 2$  C) and are preserved by the most appropriate method (From Valeri, C. R., Blood Banking and the Use of Frozen Blood Products, CRC Press, Boca Raton, Fla., 1976, 3. With permission.)

FIGURE 2

Twenty-four-hour posttransfusion survival and function of red blood cells after storage in acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) anticoagulant for as long as 21 days and in CPD plus 0.25 mM adenine for as long as 35 days. The units were stored at 4 C as whole blood or as a red blood cell concentrate with a hematocrit value of 70 to 80 V%. (From Valeri, C. R., Valeri, D. A., Dennis, R. C., Vecchione, J. J., and Emerson, C. P., Crit. Care Med., 7, 440, 1979. With permission.)

FIGURE 2

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FIGURE 3

Red cell 2,3 DPG, ATP, and inorganic phosphorus levels in red blood cells after storage as whole blood with a hematocrit value of 45 V% or as a red blood cell concentrate with a hematocrit value of 70 to 80 V% or of greater than 90 V%, in ACD or CPD anticoagulant at 4 C for as long as 17 days. Neither the units of whole blood nor the units of red blood cell concentrates with hematocrit values of 70 to 80 V% were mixed during storage. Each unit of red blood cell concentrate with a hematocrit value of greater than 90 V% was separated into two equal parts: one part was mixed during storage at 4 C and the other part was not. (From Valeri, C. R., Blood Banking and the Use of Frozen Blood Products, CRC Press, Boca Raton, Fla., 1976, 48. With permission.)

FIGURE 3

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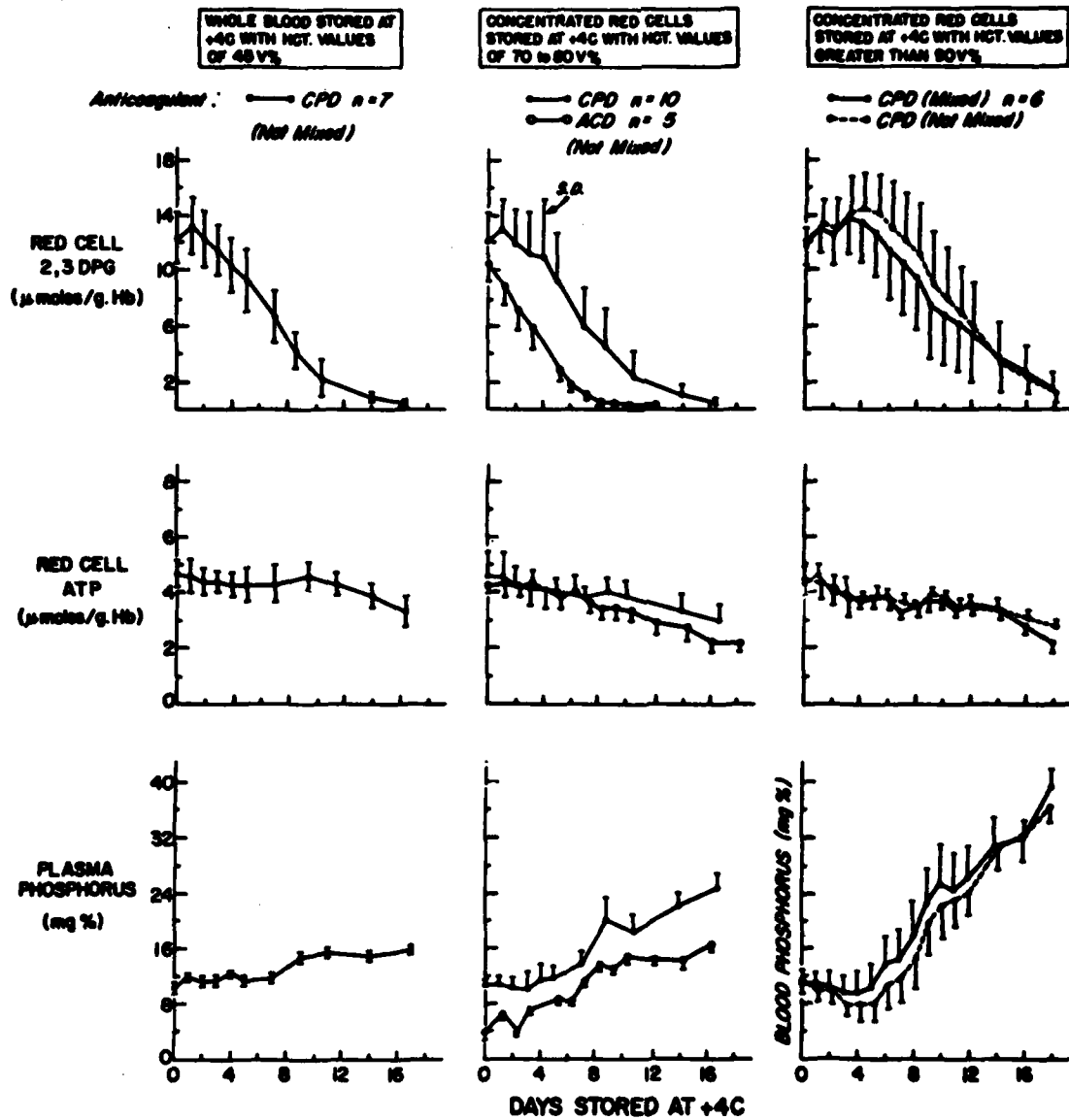


FIGURE 4

The 2,3 DPG levels of red cell concentrates stored at 4 C in  
CPD, CPDA-1, CPDA-2 or CPDA-3.

FIGURE 4  
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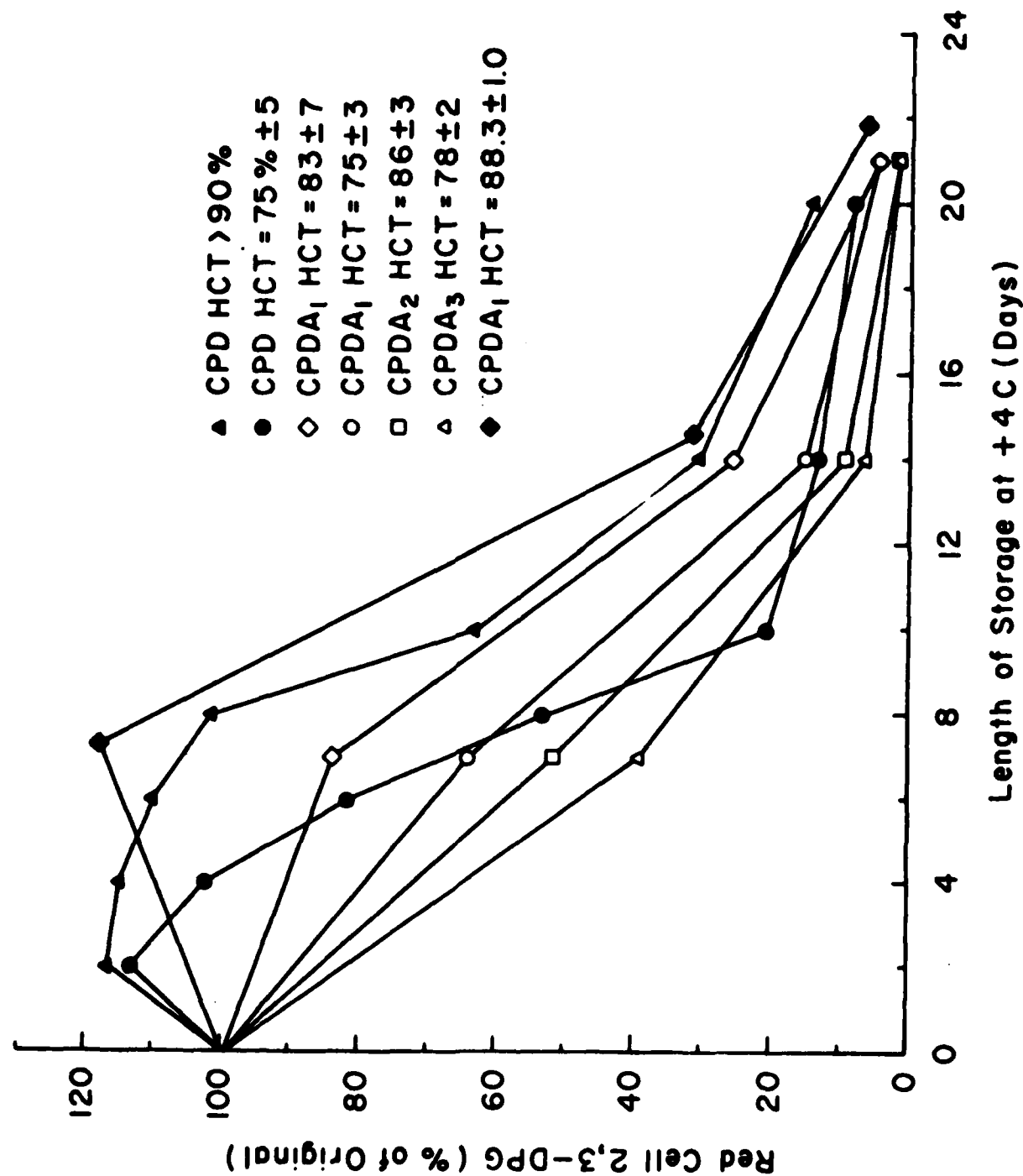




FIGURE 5

Twenty-four-hour  $^{51}\text{Cr}$  posttransfusion survival of red blood cell concentrates stored with hematocrit values ranging from 80 to 90 V% at 4 C in CPD, CPDA-1, CPDA-2 and CPDA-3 for up to 35 days.  
(From Valeri, C. R., Plasma Therapy, 2, 155, 1981. With permission.)

FIGURE 5

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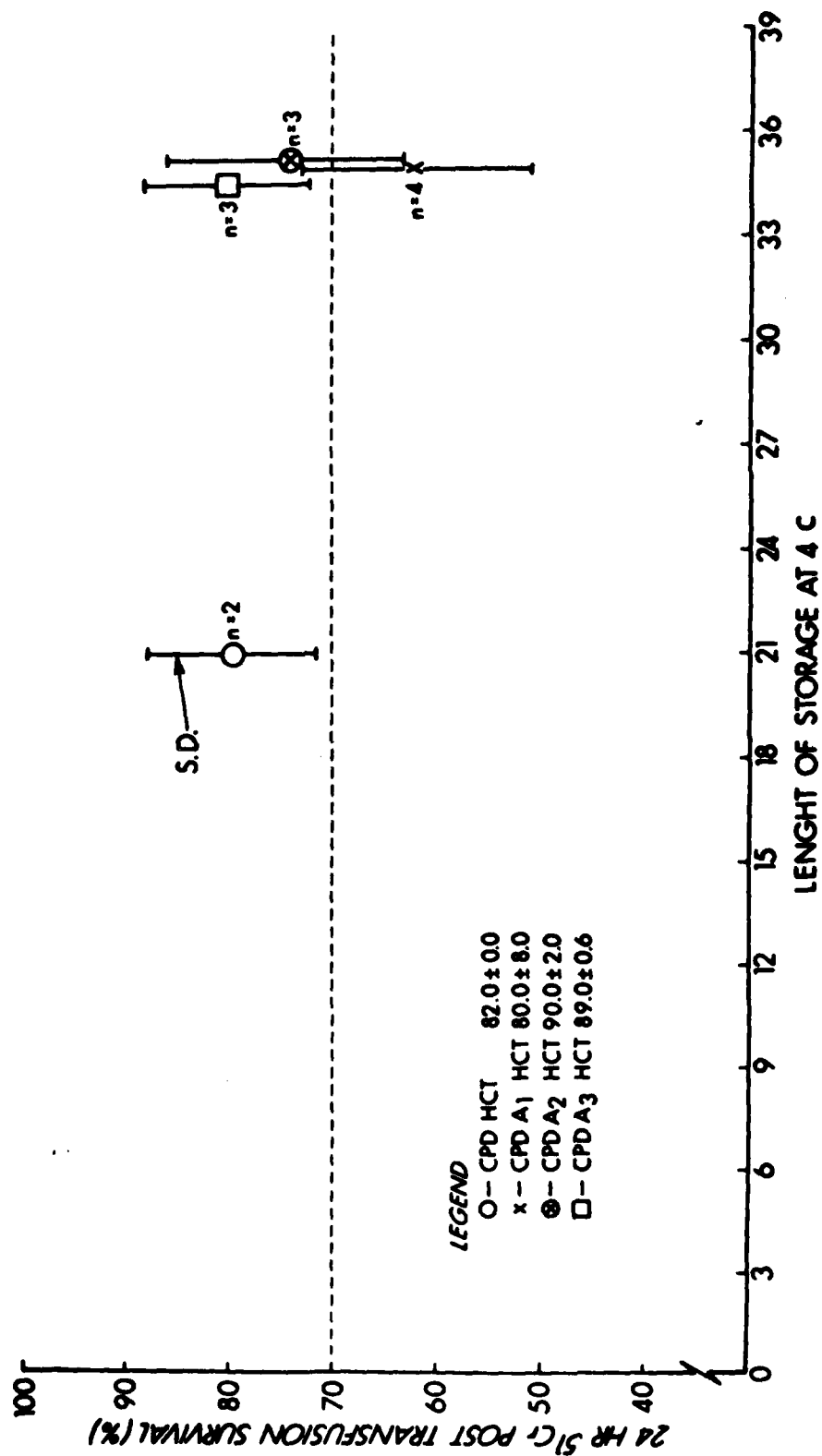


FIGURE 6

The 2,3 DPG levels in red blood cells stored in citrate-phosphate-dextrose as whole blood with a hematocrit value of 40 V% or as a red blood cell concentrate with a hematocrit value of 70-80 V% or of greater than 90 V%. Neither the whole blood nor the red blood cell concentrate was mixed during liquid storage at 4 C. (From Valeri, C. R., Surgical Rounds, 4, 41, 1981. With permission).

FIGURE 6  
VALERI C.R.

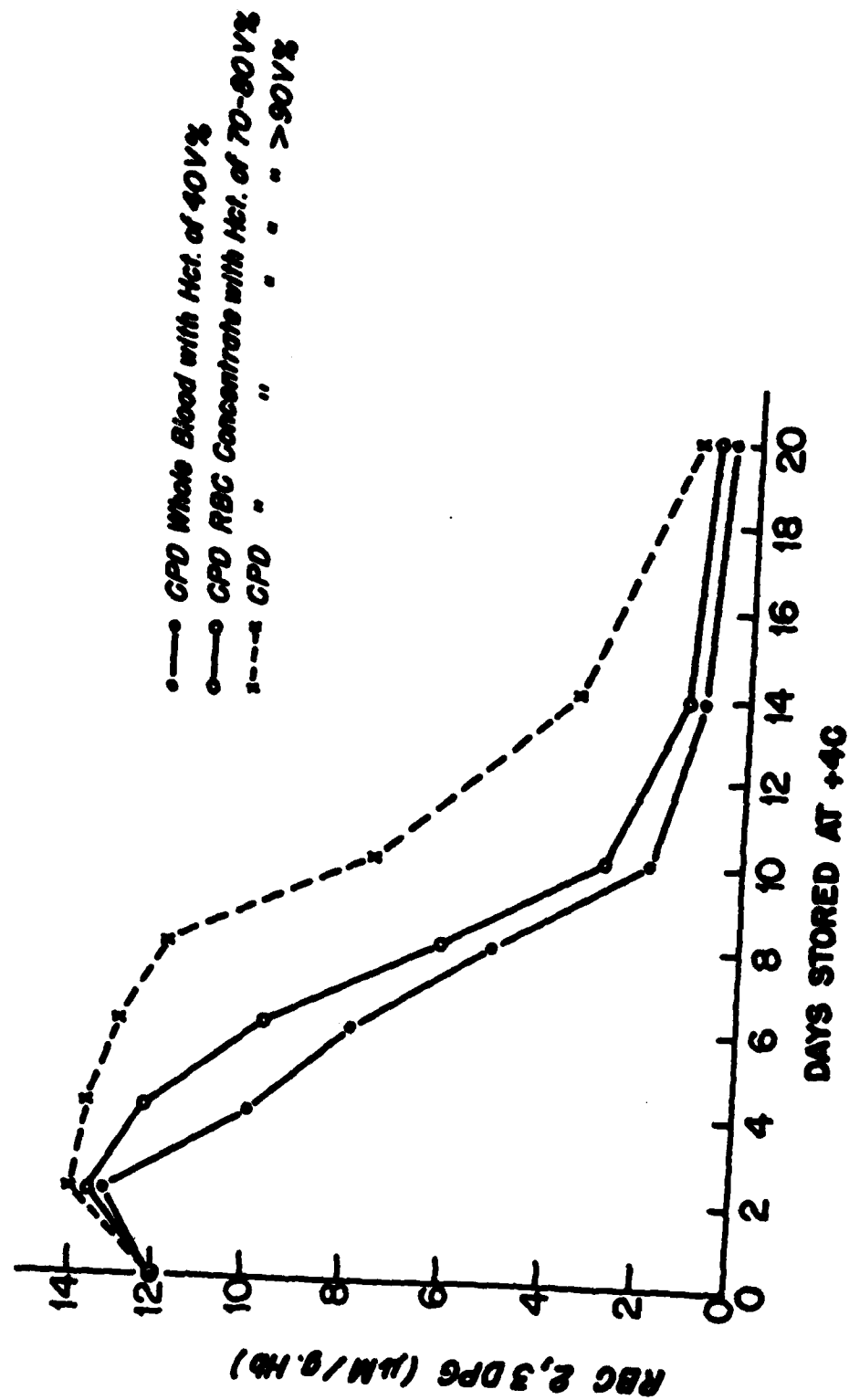
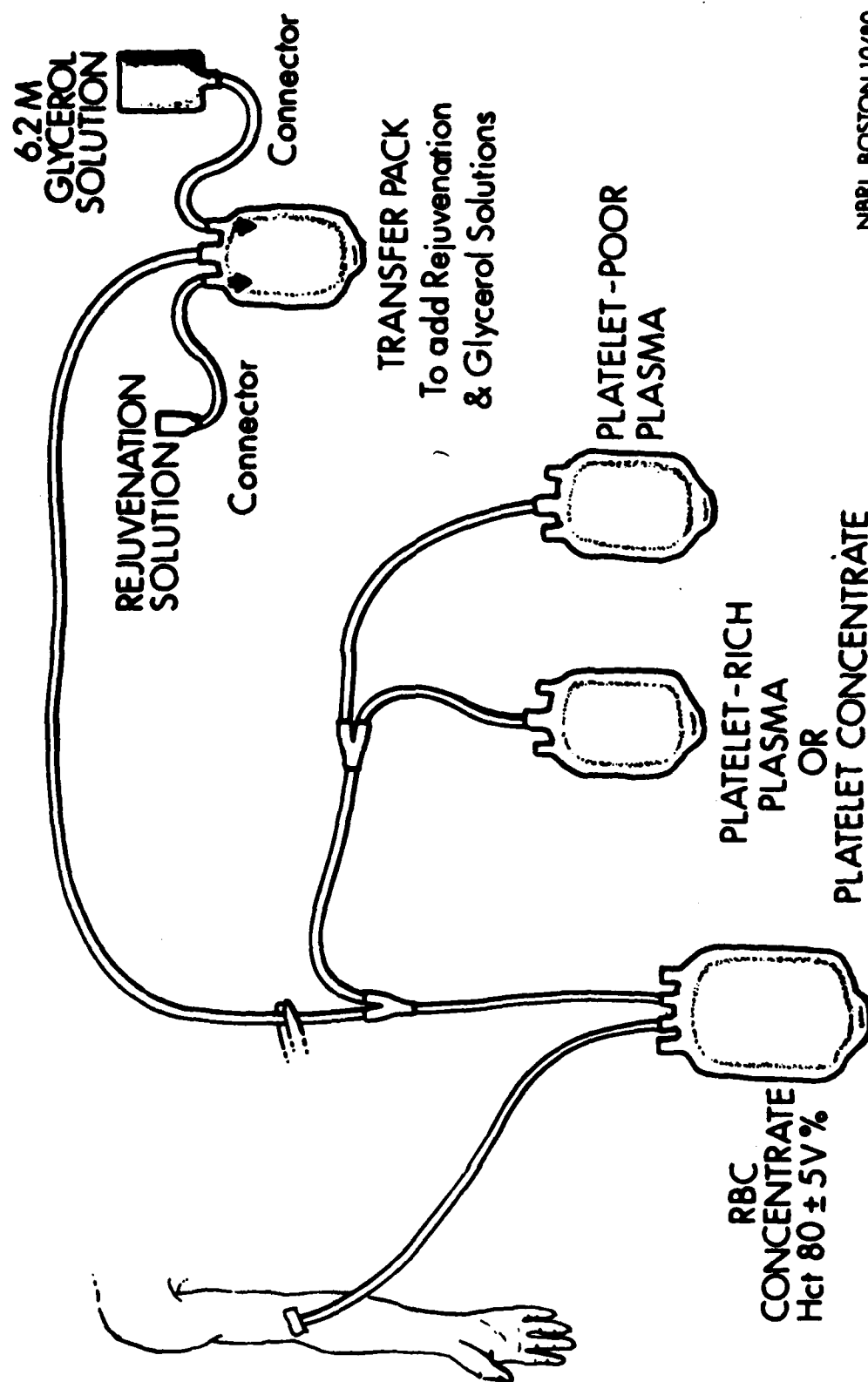


FIGURE 7

A diagram showing the quadruple PVC plastic bag system with a 600 ml primary bag. This system is used for blood collection, component preparation, biochemical modification, glycerolization and freezing. One of the integrally attached transfer packs has 2 ports through which the rejuvenation solution and the glycerol solution are added to the red cells in the primary collection bag; this same transfer pack subsequently is used for collection and discard of the supernatant solution containing the rejuvenation and glycerol solutions. (From Valeri, C. R., Valeri, D. A., Anastasi, J., Vecchione, J. J., Dennis, R. C., and Emerson, C. P., Transfusion 21, 139, 1981. With permission.)

FIGURE 7  
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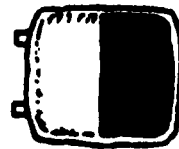
FIGURE 8

A diagram of thawing and washing of red blood cells frozen in the primary polyvinyl chloride plastic collection bag, including an outline of the three steps of the dilution of the thawed glycerolized red blood cells with 50 ml of 12% sodium chloride, 100 ml of 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus, and 150 ml of 0.9% sodium chloride-0.2% glucose-40 mg% inorganic phosphorus. After dilution, the red blood cells were washed by automated serial centrifugation in the IBM Blood Processor 2991-1 or 2991-2 or by continuous-flow centrifugation in the Haemonetics Blood Processor 115. (From Valeri, C. R., Valeri, D. A., Anastasi, J., Vecchione, J. J., Dennis, R. C., and Emerson, C. P., Transfusion 21, 142, 1981. With permission.)

FIGURE 8

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RED BLOOD CELLS  
THAWED AT 42°C  
FOR 10 MINUTES



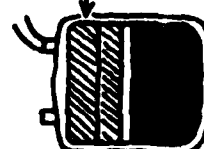
Hematocrit 65 V %



50 ml of 12% NaCl  
Added with Agitation  
& then Equilibrated  
for 2 Minutes



100 ml of 0.9% NaCl-Glucose-Phosphate  
Added with Agitation and then  
Equilibrated for 2 Minutes



150 ml of 0.9% NaCl-Glucose-Phosphate  
Added with Agitation and then  
Equilibrated for 2 Minutes

DILUTED RED BLOOD CELLS WITH  
GLYCEROL CONCENTRATION OF  
~15% W/V ARE WASHED WITH 1.5 TO  
1.6 LITERS OF 0.9% NaCl - GLUCOSE -  
PHOSPHATE

1. Automated Serial Centrifugation  
in the IBM Blood Processor
2. Haemonetics Blood Processor 115  
Using Continuous Flow, Non-Programmed

NBRL - Boston 11/78



FIGURE 9

A diagram showing a modified quadruple PVC plastic bag collection system with an 800 ml primary bag. This system is used for blood collection, component preparation, biochemical modification, and glycerolization and freezing. This modified system has an adaptor port attached to the tubing connecting the primary collection bag to two integrally attached transfer packs, and a Y connector harness, as shown, is used to add the rejuvenation solution and glycerol solution to the red blood cells in the primary bag, with the remaining transfer pack subsequently used for collection and discard of the supernatant solution containing the rejuvenation and glycerol solutions. (From Valeri, C. R., Plasma Therapy, 2, 155, 1981. With permission.)

FIGURE 9  
VALERI C.R.

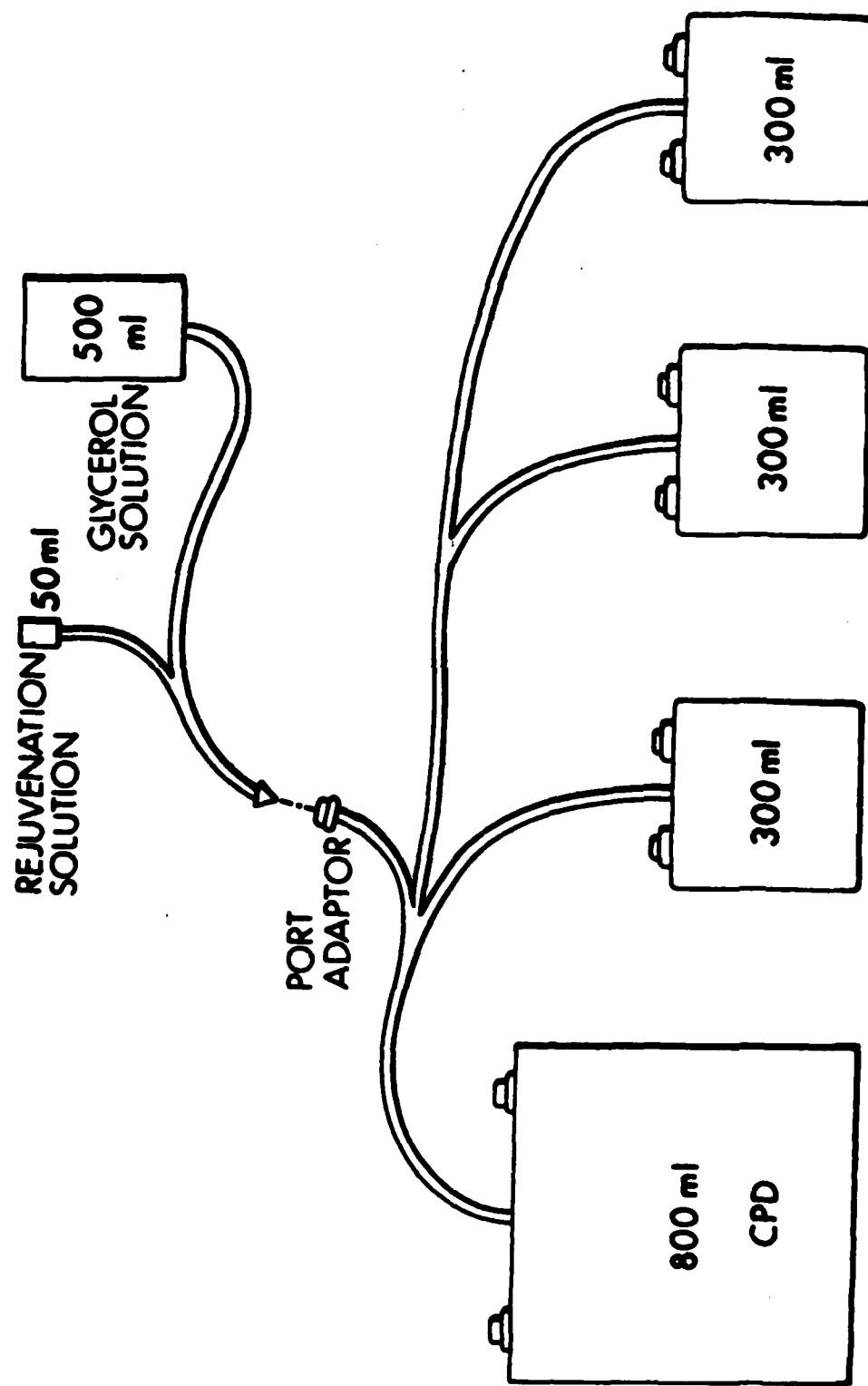


FIGURE 10

A diagram showing a modified double PVC plastic bag collection system with an 800 ml primary bag. This system is used for blood collection, component preparation, biochemical modification, glycerolization and freezing. In this modified system, an adaptor port is attached to the tubing that connects the primary bag to a single integrally attached transfer pack. A Y connector harness, as shown, is used to add the rejuvenation solution and glycerol solution to the red blood cells in the primary bag, with the remaining transfer pack subsequently used for collection and discard of the supernatant solution containing the rejuvenation and glycerol solutions. (From Valeri, C. R., Plasma Therapy, 2, 155, 1981. With permission.)

FIGURE 10  
VALERI C.R.

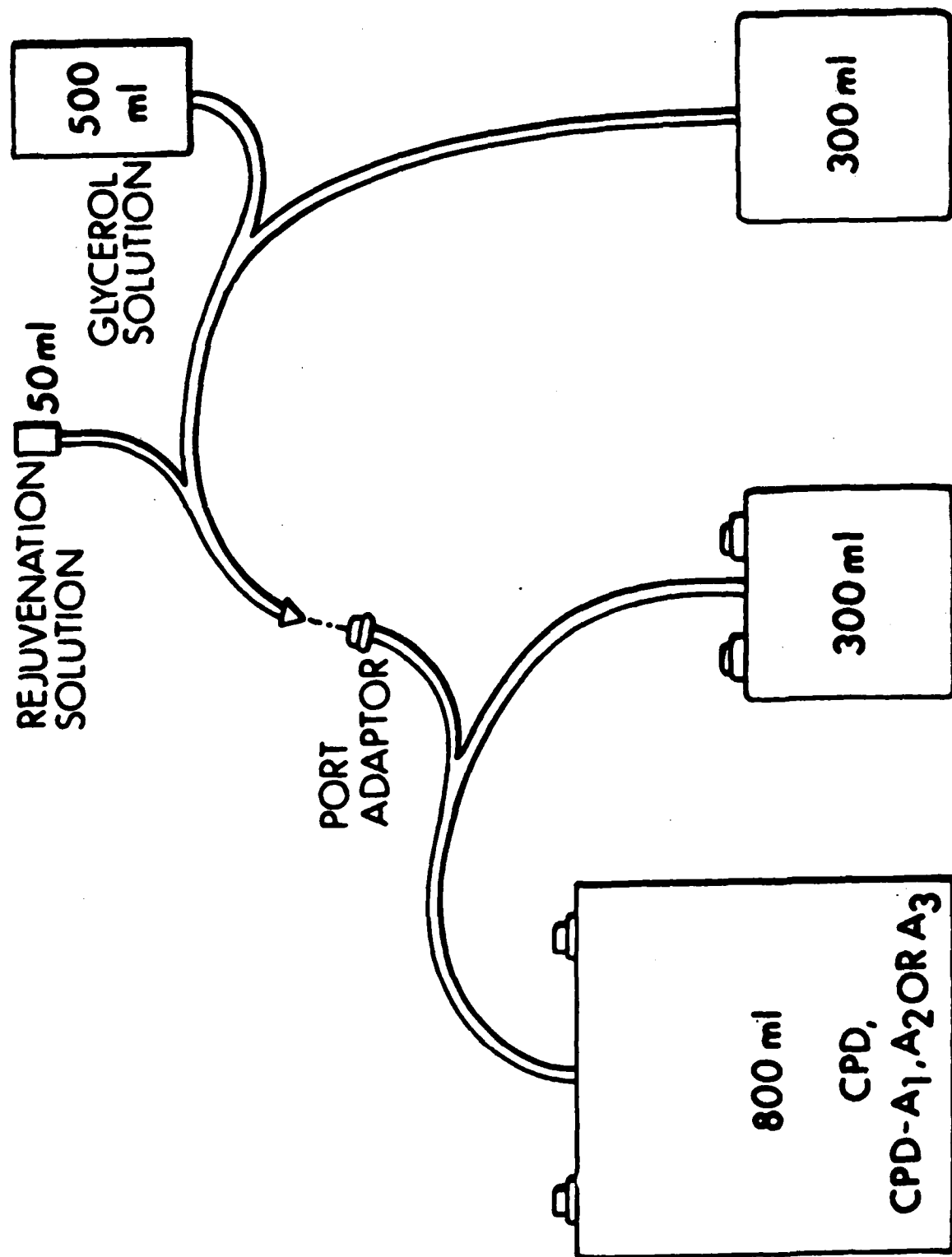


FIGURE 11

Diagram of the Frozen Blood Bank Module designed to fit into an Isocontainer whose external dimensions are 20 feet long, 15 feet wide, and 8 feet high. The space contains 2 air cooled -80 C mechanical freezers, 8 Haemonetics Blood Processor 115 red cell washers, 2 refrigerated centrifuges, 2 water baths, and 2 modified Eberbach shakers.

FIGURE 11  
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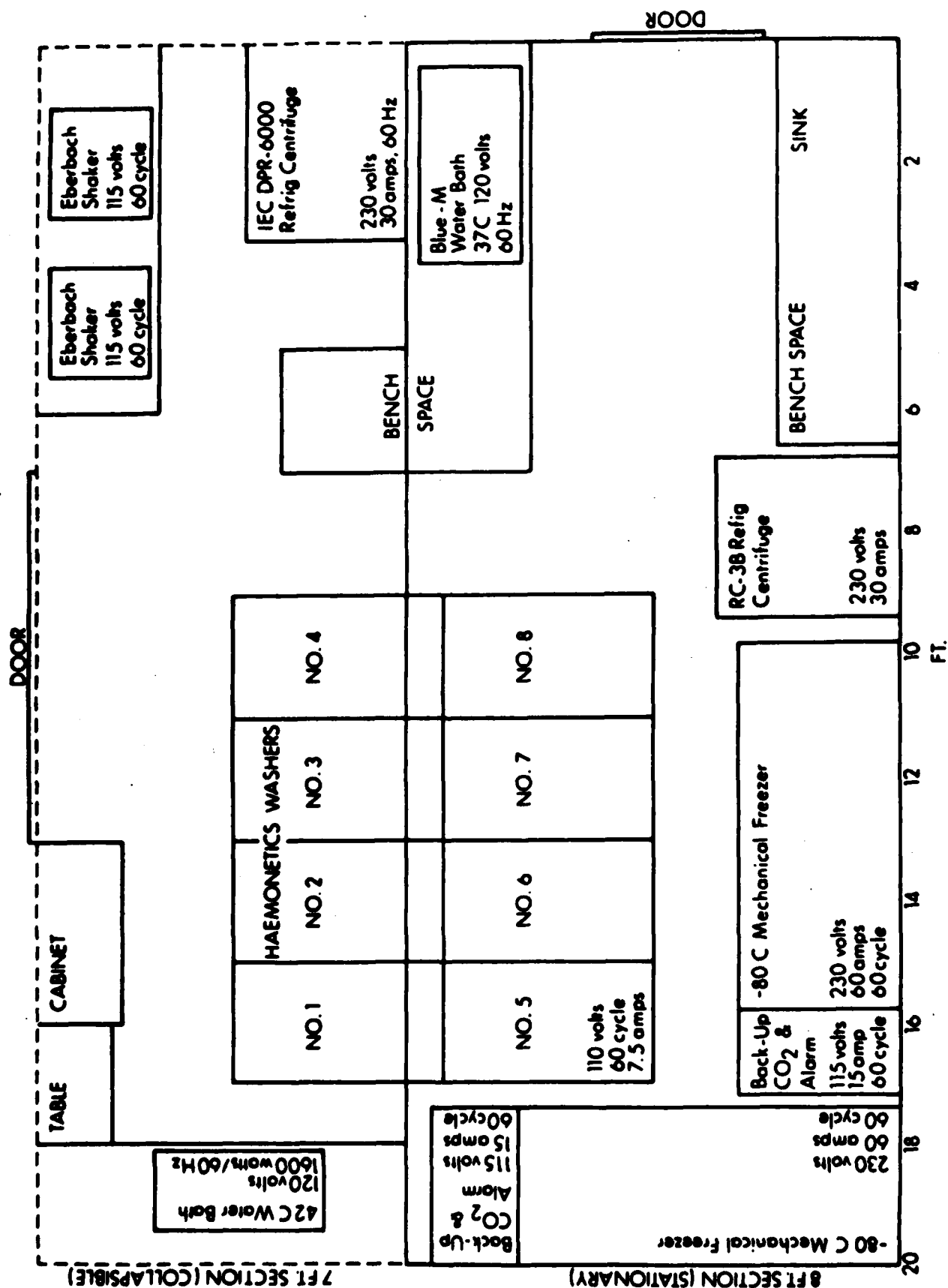


TABLE 1

CLINICAL INDICATIONS FOR FREEZE-PRESERVED RED BLOOD CELLS

1. Rare red blood cells and selected red blood cells lacking antigens that commonly sensitize patients.
2. Autotransfusions.
3. Preservation of universal donor red blood cells (O-positive and O-negative) before outdating.
4. Red blood cells with improved capacity to deliver oxygen:
  - a. Hypothermic patients in hemorrhagic shock.
  - b. Patients with fixed cerebral and/or coronary blood flow.
  - c. Use for extracorporeal circulation and hypothermia.
5. Red blood cells free of white blood cells, platelets, plasma protein and reduced amount of microaggregates.
6. Red blood cells free of citrate and vasoactive substances.

(From Valeri, C. R., Valeri, D. A., Dennis, R. C., Vecchione, J. J., and Emerson, C. P., Crit. Care Med., 7, 444, 1979.  
With permission.)

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